Infectious Allergy

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• The allergic reactivity which accompanies various infectious diseases is different in certain fundamental principles from the allergic disease associated with hypersensitivity to such agents as pollens, dust, and foods. Allergic sensitivity associated with tuberculosis comes about because of the participation of a fatty fraction of the bacillus with another component of the bacterium which is actually the sensitizing substance. The fatty fraction, if isolated from the bacillus, can act with various kinds of sensitizing substances that have nothing to do with tuberculosis to bring about the same kind of hypersensitivity that accompanies tuberculosis. Attempts are being made to learn more about the manner of action of this factor, and also to find out whether the organisms of other infectious diseases may have similar chemical constituents that cause allergic disease.

A GENERAL STUDY of various immunological facets of experimental tuberculosis which has been carried on in the Department of Bacteriology and Experimental Pathology at Stanford University during the past eight years led to the trail of tuberculous hypersensitivity, and subsequently into the bypaths of allergic sensitivity to other infectious diseases. From those investigations have come certain factual information and some speculation about fundamental factors that may be responsible for these hypersensitive states.

Bacterial allergic reactivity is a manifestation of the group of hypersensitivities termed "delayed," and these are distinguished by certain important characteristics from other hypersensitive states which are called "immediate" and which are exemplified by anaphylaxis, Arthus reactivity, and the atopic states. Differences between these two broad categories extend to the *induction* of the respective reactive states as well as to the *reactivity* of sensitized subjects.

With regard first to induction, it is well known that infectious hypersensitivity requires for its establishment the presence in the tissues of something in addition to the antigen responsible for it. In all instances in which the sensitizing antigen has been chemically isolated from an infectious agent this antigen acting by itself has been found inadequate for the induction of infectious hypersensitivity in normal subjects. Consequently, over the years it has come to be generally recognized that infectious allergic reaction cannot occur save in the presence of the infectious agent itself in the body, albeit this need not necessarily be living. In contrast, it is well known that any soluble or insoluble antigenic substance can give rise to the immediate type of hypersensitive state.

With respect to the reactivity of sensitized subjects, there are several features which distinguish these two states. First, the chronological sequence of events following the local or systemic application of antigen differs; from this fact, of course, the two categories have derived their names. Secondly, it is well known that humoral antibodies are intimately associated with the immediate hypersensitive state whereas no relationship between antibodies of blood or tissue fluids has been established for delayed reactivity. Consequently, delayed reactivity cannot be transferred to normal subjects by serum, while immediate reactivity can be so transmitted. (Certain cells, on the other hand, can transfer both forms of reactivity.[3,7,8,9,10]) Finally, the cellular responses in delayed hypersensitivity are more catholic than are those in the immediate type. So far as is known, all cells of the body with bacterial allergic sensitivity are capable of responding to the proper bacterial antigen; recent evidence indicates that this may extend even to the erythrocytes.[16] On the other hand, in immediate hypersensitivity the responsive units seem to be restricted to smooth muscle, blood vessel endothelium, collagen, and possibly mucus-secreting cells such as those of the respiratory tract. In a "geographical" sense of course this means that practically any part of the body may become involved in a hypersensitive response of the immediate kind, but the cellular basis for this is more limited than that which underlies delayed reactions.

These various differences provide criteria which may be applied to the controlled study of allergic reactivity. The factor of time necessary for the elicitation of the reaction, for example, provides a simple,

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but rather subjective, basis for distinction. A more objective ground is supplied by the study of the capabilities of humoral antibodies of the hypersensitive subject to transmit reactivity. Perhaps the best objective evidence, however, comes from a study of the cells able to react to exposure with antigen. If tissues from subjects with immediate reactivity are isolated for studies of this kind *in vitro* or if observations are carried out *in vivo* as has been done by Abell and Schenck,[1] it is observed that only those cells and tissues mentioned above visibly take part in the reactions to antigen. If, however, tissues without smooth muscle are explanted, then the circumstances are propitious only for the demonstration of the delayed form of reactivity, for in the absence of vascular elements or smooth muscle, nothing visible happens. If changes in collagen can occur in these circumstances, they have not been described. Studies of the reactions of cultures of bone marrow, spleen, and other tissues have consequently played an important part in the development of present knowledge of the reaction mechanism in hypersensitivity. The two photographs in Figure 1 illustrate the point. The picture on the left is of the bone marrow of a guinea pig which had been brought to a high level of anaphylactic sensitivity by the injection of protein obtained from the tubercle bacillus. (Any other soluble antigenic substance would have served as well for this demonstration.) The point is that this animal had pronounced Arthus reactivity to the intradermal injection of the protein and, several days before this fragment of tissue was taken, had pronounced anaphylactic symptoms following the injection of a minute amount of the antigen into the bloodstream. The photograph was made after the bone marrow cells in culture had been subjected to the presence of the protein for 24 hours. These cells are viable and motile; they are entirely unaffected by the presence of the antigen to which the animal itself was highly sensitized. In contrast, the photograph at the right reveals the situation under exactly the same experimental circumstances except that the bone marrow was removed from an animal sensitized to the tubercle bacillus. The allergic reactivity of this animal is directed against precisely the same protein of the bacterium, but in this case it is obvious that the cells of the marrow are so highly responsive to the presence of the protein antigen that death of most or all the cells has occurred.

An analogous but more simply performed objective criterion to permit the distinction between immediate and delayed hypersensitivity is furnished by the use of the cornea of the sensitized animal for observations of reactivity. As the cornea is without

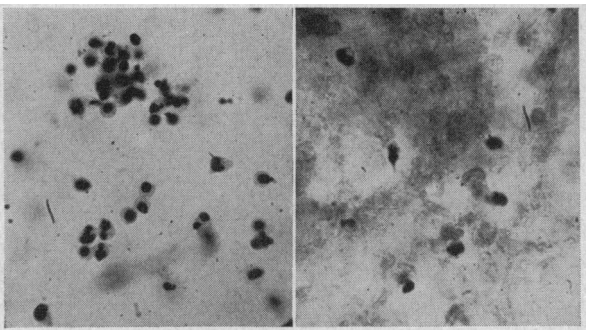

Figure 1.—*Left*—Bone marrow culture from guinea pig sensitized to tuberculoprotein. These cells have been exposed to the antigen for 24 hours and remain unaffected. (Hematoxylin and eosin stain, × 900.) *Right*—Bone marrow culture from tuberculous guinea pig after exposure to tuberculoprotein for 24 hours. Extensive cellular destruction has occurred. (Hematoxylin and eosin stain, × 900.)

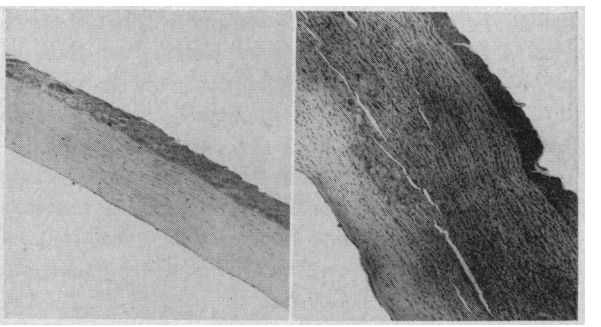

Figure 2.—*Left*—Cornea of guinea pig sensitized to tuberculoprotein. Forty-eight hours earlier a droplet of antigen had been injected, but no evident injury resulted. (Hematoxylin and eosin stain, × 85.) *Right*—Intense inflammation of cornea of tuberculous guinea pig 48 hours after injection of tuberculoprotein. (Hematoxylin and eosin stain, × 85.)

blood vessels or smooth muscle, it is incapable of entering into a reaction of the immediate type. If a small droplet of antigen is injected into the cornea of an animal with anaphylactic hypersensitivity and the tissue is observed for the following one or two days, no change is noted beyond the slight trauma incident to the injection itself. In contradistinction, an injection of the responsible antigen into an animal with delayed hypersensitivity leads, in 24 to 48 hours, to the development of striking keratitis. As is to be expected, if vascularization of the cornea is induced by treatment with some nonspecific irritant in advance of the test, then it is possible to evoke a corneal response in the anaphylactic animal also. Figure 2 illustrates these points. The photograph to the left in Figure 2 is of a section of the cornea of a guinea pig with a high level of anaphylactic reactivity to tuberculoprotein. The preparation was made two days after the injection of antigen into the cornea, and there was no detectable change in the tissue. In contrast to this, the

photograph to the right is of the cornea of an animal with delayed reactivity to the same antigen as the result of tuberculous infection; the reaction was intense and probably was accompanied by destruction of corneal cells. The two cases illustrated in Figure 2 are entirely analogous to the two illustrated by tissue culture photographs in Figure 1.

The allergic reactivity of tuberculous infection is amongst the most striking of the hypersensitive states accompanying infectious disease. It has been known for some years that the remarkable responsiveness which the body may develop to this bacillus is actually directed against the easily separated proteins of the organism. This fact was established upon a firm basis some years ago when Seibert[17] successfully crystallized a protein of the microbe and demonstrated its ability to elicit responses in tuberculous subjects, and it is substantiated daily through the use of purified protein derivative of the bacillus for clinical skin testing. Although this protein is a potent antigen its injection into normal animals does not eventuate in the development of the hypersensitive state characteristic of the disease itself. The humoral antibody response is good, and immediate hypersensitive reactivity of a high order becomes readily demonstrable in animals treated in this fashion.

For some years the question has been this: What distinguishes the antigenic activity of this protein as it exists in the body of the tubercle bacillus from that which it shows as an isolated substance?

Several years ago, during the course of experiments in which attempt was being made to evaluate the responses of the body to various isolated components of the tubercle bacillus as well as to the bacterial body deprived of various of its constituents, it was noted that animals which had been repeatedly inoculated with bacilli from which lipoidal substances had been extracted with ether, alcohol, and chloroform, did not become reactive to old tuberculin. This result was striking because it was apparent from the same experiment that the protein of these defatted bacilli had not lost its antigenic properties, since high levels of antibody developed in the treated animals and they became anaphylactically sensitized. In the same experiment, there were groups of animals which had received bacilli that had been only partially extracted; thus, one group had been treated with microbes from which only the ether-alcohol soluble substances had been removed, while another received organisms extracted only with chloroform. It was noted that the group which had received the ether-alcohol extracted bacilli became sensitized, while the animals vaccinated with the chloroform-extracted bacteria did not become sensitized. It was already known from the work of Anderson[2] that ether-alcohol removes phospholipids from the tubercle bacillus, whereas chloroform extracts a waxy substance composed principally of mycolic acid, a long chain fatty acid, alone and in combination with a carbohydrate. It appeared, then, that this waxy substance must be concerned in the induction of tuberculous hypersensitivity to the protein of the bacillus. This inference was soon substantiated by the injection into animals of the isolated lipoidal factor along with the isolated protein. This resulted in the establishment of hypersensitivity to tuberculin, and the delayed nature of this hypersensitivity could be demonstrated by all the various criteria which have already been described. No other combination of bacterial elements imitated this effect. Since that time the portion of the waxy lipid concerned in this activity has been more definitely delimited. It appears to be the mycolic acid-polysaccharide ester portion, but certain recent additional findings have suggested that perhaps something even simpler than this may be concerned, the chemical nature of which has not yet been ascertained.[11, 12, 13] Meanwhile, Choucroun who had also been engaged in work with bacillary fractions came to the conclusion that a factor which she termed Pmko is concerned in infectious hypersensitivity. This factor has been shown by her to be the mycolic acid-polysaccharide complex.[4, 5]

At Stanford, studies have been carried forward in two directions to investigate the possibility that the phenomena observed in connection with tuberculous hypersensitivity might also be present in other allergic conditions. First, it was of interest to know whether other antigens than the tuberculoprotein might serve along with the waxy lipid to induce the delayed type of hypersensitivity in animals. This was found to be the case with two antigens tested. one a well-known protein, egg albumin, the other a simple substance which has the capacity to induce contact reactivity when injected into the skin, picryl chloride. In the case of the albumin, it was found possible to establish hypersensitivity analogous in all respects to that obtaining in tuberculosis. With picryl chloride, contact reactivity was induced by means of intraperitoneal installation of the chemical along with the lipoidal element. Picryl chloride when injected into the peritoneal cavity alone does not act as an incitant of contact reactivity.[14, 15]

The other line of investigation has been concerned with the possibility that there might exist in infectious agents other than the tubercle bacillus a similar

relationship between a lipid and an antigen in inducing infectious allergy. For this purpose three organisms were chosen, one to represent the bacteria, one the viruses, and one the fungi. A group of C hemolytic streptococcus has served as the bacterium, vaccinia as the virus, and *Aspergillus niger* as the fungus. In each of these cases the allergy of infection reveals itself through tests with isolated proteins obtained from the respective agents. Some difficulty has been encountered in determining whether or not there may be a lipoidal factor analogous to that of the tubercle bacillus in these various agents, but so far evidence has been elicited that this may be the case in the streptococcus and the virus, although it is not conclusive enough to warrant publication of details. Results of work with the fungus cannot yet be interpreted.

Since these phenomena began to come to light, one concern of the investigators has been the question of the mechanism through which the lipoidal factor may modify the body so that it responds with delayed hypersensitivity to a protein antigen. So far as can be determined, the lipoidal factor itself has no antigenicity. The reactivity of the body is directed, as was mentioned previously, against the protein. The lipoidal factor possesses the ability to induce the formation of epithelioid and related cells, and it has been thought that these may be associated with the infectious hypersensitive response of the body. However, other portions of the bacillus which are not concerned in this hypersensitivity can also call forth such cells. It has been assumed that the lipoidal factor may in some way modify various cells so that they are more amenable to the entry of antigens than they would ordinarily be. For the purpose of studying this point, the tuberculoprotein used has been marked by the introduction of a bright colored dye into the molecule, in the hope that it might thus be possible to trace the distribution of antigen in the body under the influence of the waxy factor. So far the method has given no clue to support the hypothesis, but this may be owing to fault in the technique rather than to error in the hypothesis. It is difficult to see this dye in small amounts in the tissue. For this reason attempts are being made to trace the antigen by using a fluorescent compound such as is being employed by Coons[6] for studies of the distribution of antigens in the body.

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